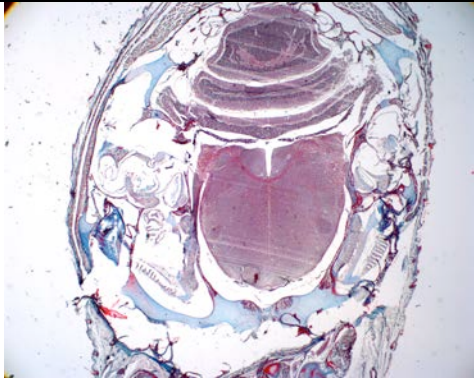
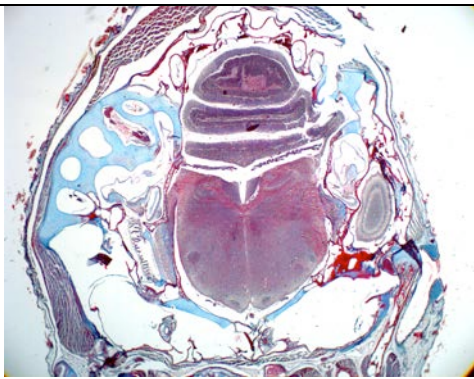


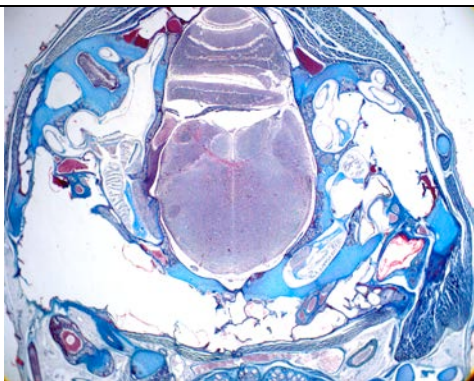
Images taken from quail samples submitted to Dr. Fermin laboratory for analysis, including STS-79 samples. Parts of the embryos returned from the STS-79 flight were shared among many PI, and thus were not available for whole head analyses shown here. Since the samples returned and available after sharing, were only a few pieces of temporal bone the PI includes here representative samples for laboratory, synchronous and flight quails from MIR samples submitted prior to the STS-79 flight but treated equally as the STS-79 samples.



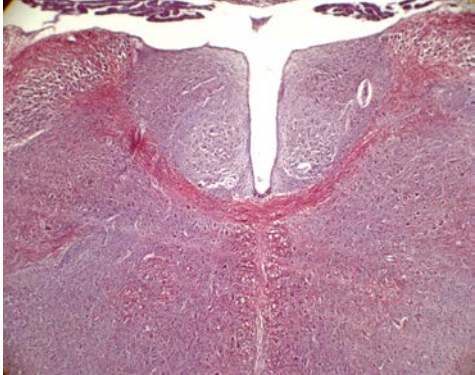
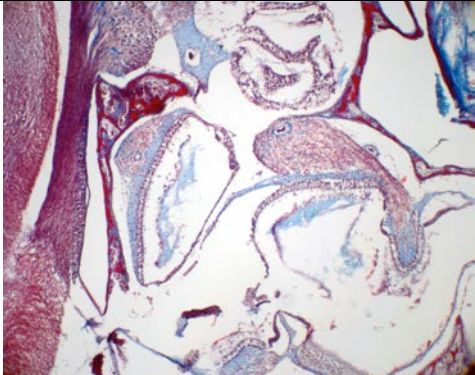
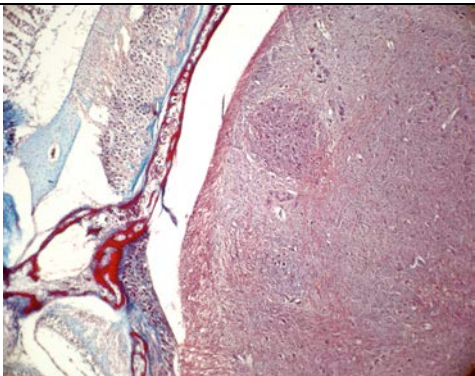
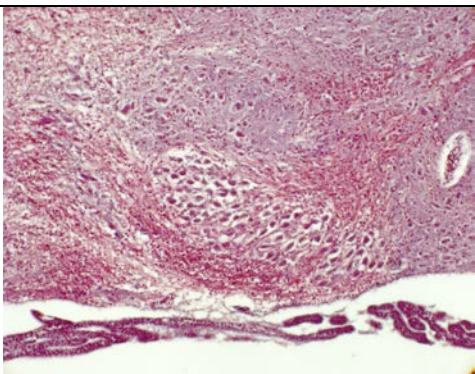
CD-FSLM-1001 (LABORATORY): This is a x2 of the hatch synchronous control. This is from a trichrome stain section of a specimen received in Dr. Fermin's laboratory. The intact brain cerebellum and inner ear compartment are seen laterally and symmetrically. Folding of the conduits of the inner ear is also observed due to inherent problems with infiltration of the ear and fluid filled cavities of the avian inner ear.



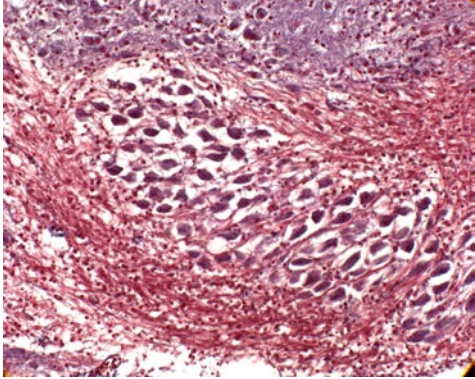
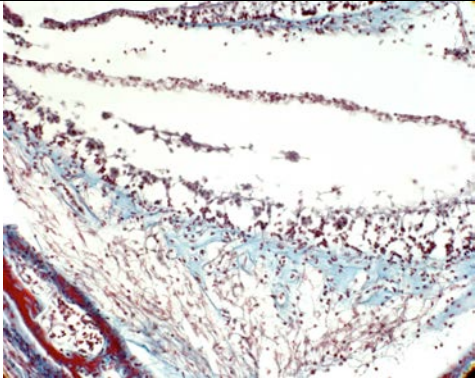
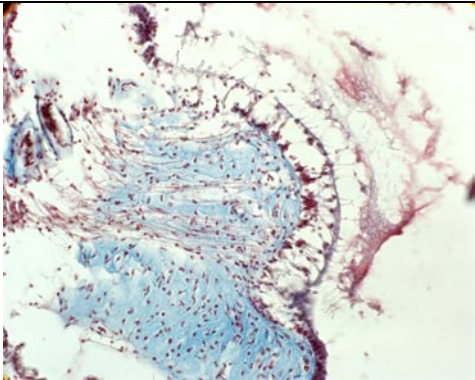
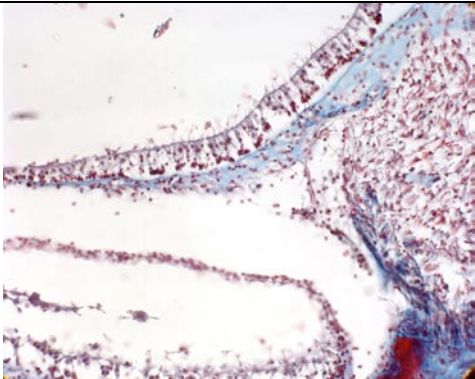
CDF-SLM-1002 (LABORATORY): This is hatch laboratory control. In this section, the compact brain and the entrance of the eighth nerve can be seen with portions of the lateral brain areas on the right hand side. The cerebellum is also apparent and part of choroid plexus can be seen. On the left side, cross section of all the utricle is evident, and convergence of portions of the superior semicircular canal as it joins the ventricle and the saccule is seen of the left of the section.

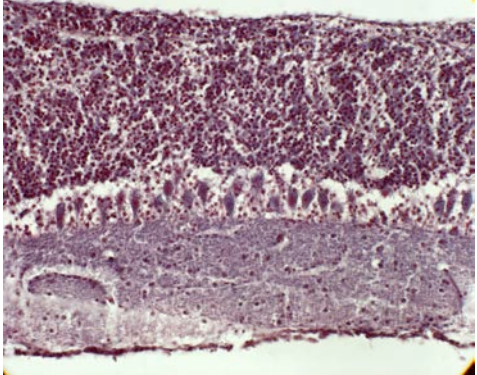
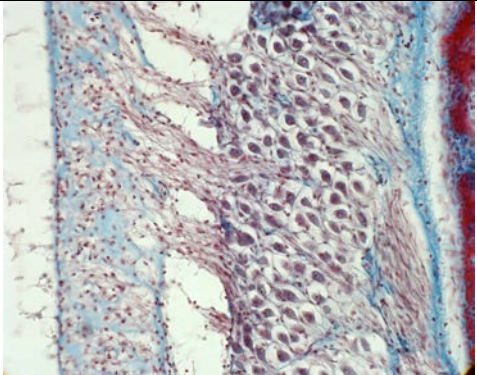
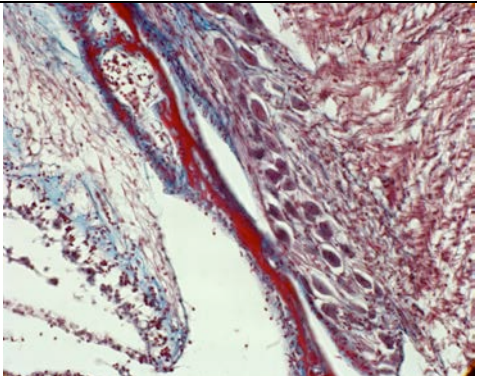
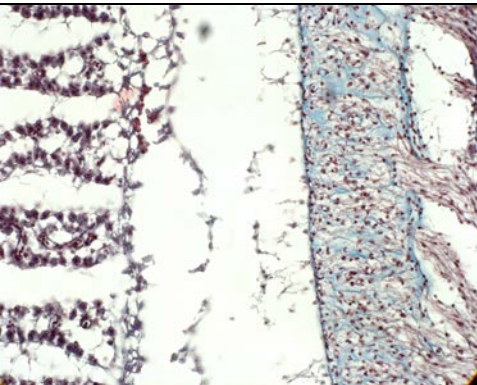


CDF-SLM-1003 (FLIGHT): This image is low objective view of a slide hatch quail. Approximately similar level as CDF-SLM-1002 showing a compact brain cerebellum, choroid plexus, and symmetrical inner ear structures. The auditory and vestibular nerve entrance to the brain stem are clear on the left side of the section.

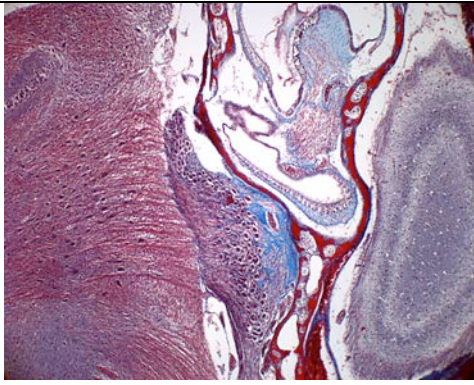
	<p>CDF-SLM-1004: (SYNCHRONOUS): This is a x10 objective view of the hatched synchronous control. Section also shows the mid-brain stem area with the superior, medial, vestibular nucleus and crossing fibers. The fourth ventricle and portions of the choroid plexus are seen. Neurons and glia are well preserved, and the descending pathway bundles fibers in the mid-line are well preserved as well.</p>
	<p>CDF-SLM-1005: (SYNCHRONOUS): This is a x10 objective view of the inner ear structures proximal to the entrance of the eighth nerve. The trunk of the vestibular nerve is seen in a branch that innervates the basilar epithelia (auditory is also clear). The tegmentum vasculosum, the sensory areas of the utricle and saccule appear poorly fixed. The majority of the cytoplasmic component was extracted during processing.</p>
	<p>CDF-SLM-1006 (SYNCHRONOUS): This is another x10 view of the synchronous control showing the bipolar neurons of the auditory and the vestibular branch of the eighth nerve adjacent to the brain stem area with the vestibular nuclei. The overall topography of the vestibular central pathway and the temporal bone are evident. Folding of the bone capsule occurred during processing, a common problem in paraffin embedding due to the many spaces of the avian skull that are filled with air and/or lymph the labyrinth.</p>
	<p>CDF-SLM-1007 (SYNCHRONOUS): This is a x20 objective view of the superior vestibular nucleus of the mid-brain stem area showing extraction of the cerebral matrix around the neurons. This is typical of poor fixation and extraction during processing due to weak cross-linking of the proteins that make up the cerebral parenchyma.</p>



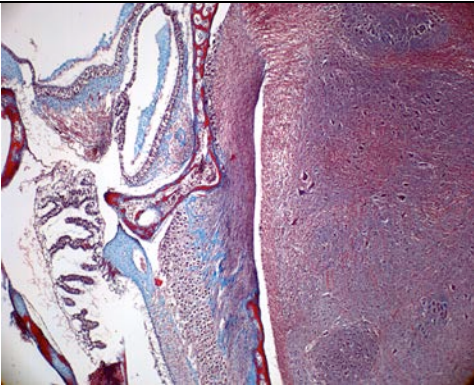
	<p>CDF-SLM-1008 (SYNCHRONOUS): This is a x40 objective view of the shrinkage of the neurons that led to the space around the cell body seen in the previous image is obvious, and the extraction of material from the periphery is evidenced in this preparation. This is direct result from lack of either penetration of the fixative or dilution of the solution, and thus poor cross-linking of the proteins.</p>
	<p>CDF-SLM-1009 (SYNCHRONOUS): This is a x40 objective view of the saccule. View of the saccule showing complete extraction of the sensory epithelial and of supporting cells. The neuropil of the saccular branch of the eighth nerve is also affected and the supporting elements that normally separate the endolymph from the perilymph are also affected.</p>
	<p>CDF-SLM-1010 (SYNCHRONOUS): This is a x40 objective view of the posterior crista of the posterior semicircular canal showing extraction of most of the support cells and part of the hair cells. Only the cuticular plate and lamina, which are generally made of filaments, remains attached to the remnants of the cupula. The cupula is made mostly of mucopolysaccharides and thus is resistant to extraction due to lack of or poor cross-linking of the primary fixative. The bluish color corresponds to the hyaline cartilage, which encases the nerve fibers, and because of this reason, some of these fibers are better preserved than those in the saccule where they are supported by hyaline cartilage that is as</p>
	<p>CDF-SLM-1011 (SYNCHRONOUS): This is a x40 objective view of portion of the utricle showing extraction of the supporting cells of the sensory epithelia, but remnants of the hair cells are still left of this sensory linear acceleration organ. A few hair cells, cuticle, and stereocilia remained in place, probably because they have high content of filaments and cytoskeletal structure that supports and prevents extraction. A few scattered otoconia are also seen. The basal membrane attached and resting on the hyaline cartilage is also seen.</p>

	<p>CDF-SLM-1012 (SYNCHRONOUS): This is a x40 objective view of a lobular of the cerebellum showing granular cells layers and Purkinje cell layer with extraction of the matrix around the cell body. Nerve recognizable structures that are usually found synapsing to the Purkinje cells are missing in this preparation. Note that the granular cells layer is mostly populated by the remnant nuclei of the cells and that most of the cytoplasm has been extracted</p>
	<p>CDF-SLM-1013 (SYNCHRONOUS): This is a x40 objective view of the auditory bipolar neuron fibers entering the auditory organ of the quail which is called basilar papilla shown here over hyaline cartilage upon which the sensory cells that rest. Notice that there is complete disappearance of the sensory and supporting cells above the basal membrane of the blue color, which represent the hyaline cartilage. The nucleus of the neurons is shrunken and the nucleoli are condensed. In addition, notice the space between the satellite cells and the body of the neurons, which is indicative of a tremendous amount of shrinkage and extraction of material around the cell body.</p>
	<p>CDF-SLM-1014 (SYNCHRONOUS): This is a x40 objective view of the vestibular branch of the nerve and bipolar neurons entering the brain stem and innervating portions of the saccule. Contrary to the auditory branch, the vestibular neurons are found outside the otic capsule and generally are better preserved than the auditory neurons. Nevertheless, extraction around the neurons is evident. Almost complete extraction of the sensory area in the saccule is obvious.</p>
	<p>CDF-SLM-1015 (SYNCHRONOUS): This is a x40 objective view of the auditory basilar epithelia and the overhanging tegmentum vasculosum. Similar to the choroid plexus, the tegmentum vasculosum is highly secretory structure composed of light and dark cells. <u>Light cells are extremely sensitive to osmotic shock and as can be seen in this image, there are no light cells remaining, only dark cells are visible.</u> In addition, no sensory structure is obvious on top of the base membrane limited by the blue color of the hyaline cartilage. These are typical changes of extraction during processing due to poor fixation and weak-cross-linking of proteinaceous components of cells and tissues.</p>

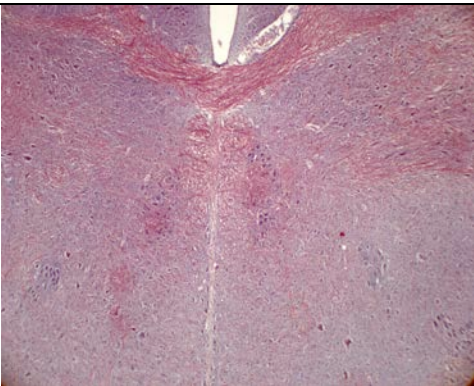




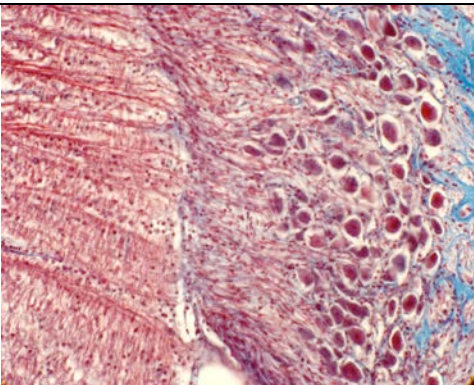
CDF-SLM-1016 (LABORATORY): This is a x10 objective view of the laboratory control showing the entrance of the vestibular nerve to the brain stem, portions of the tangential nucleus, the nucleus laminaris to the left of the epithelia of the posterior crista. At this low magnification, the inner ear structures appear well preserved. However, at higher magnification cytosol extraction is obvious.



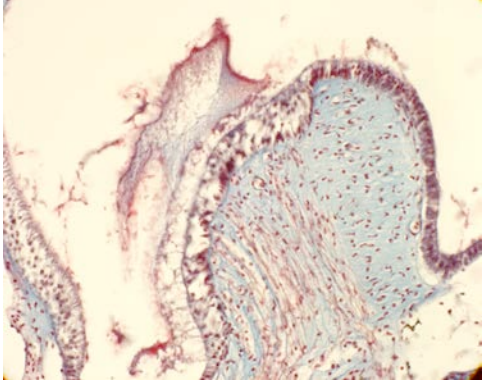
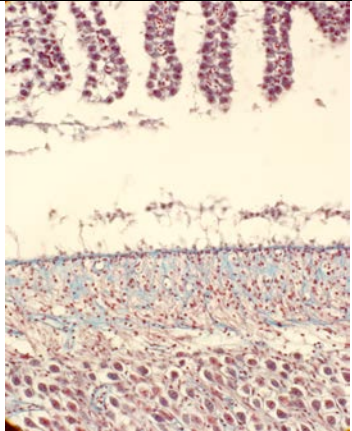
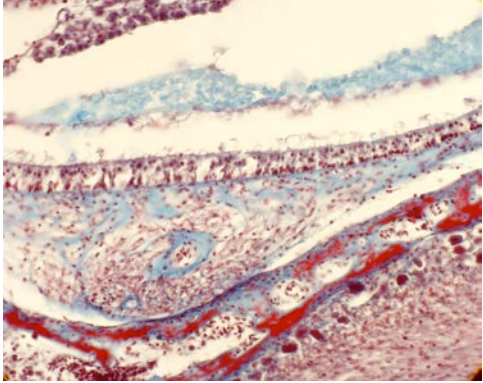
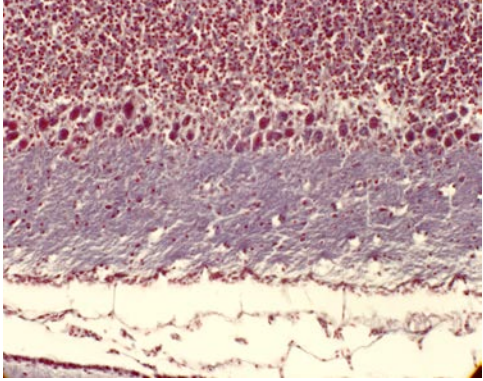
CDF-SLM-1017 (LABORATORY): Opposite side of the lab control showing entrance of the auditory nerve to the brain stem, with bipolar neurons and the sacculle with otoconia staining blue; and little extraction of the supporting cells of the saccular epithelia. The tegmentum vasculosum and the basal portion of the auditory epithelia are seen on the left bottom corner of this view.



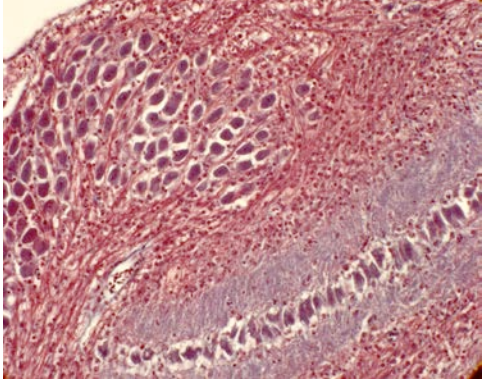
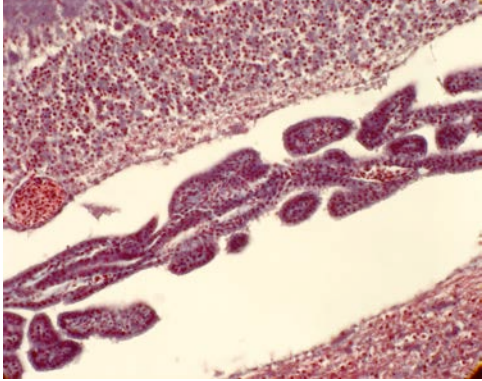
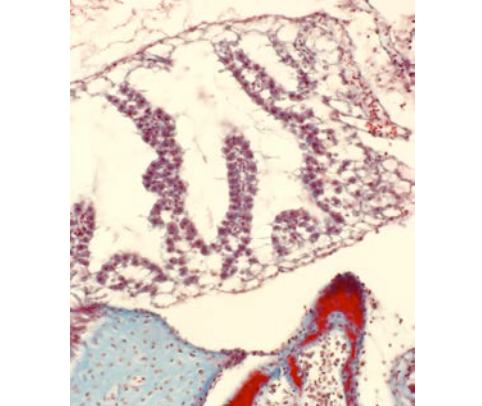
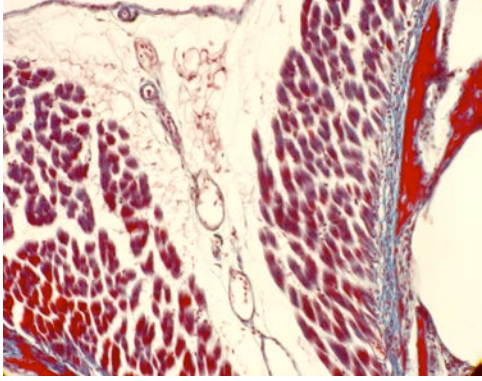
CDF-SLM-1018 (LABORATORY): Mid section of the brain stem of a lab control, x10 objective view showing fiber crossing the mid-line at the floor of the fourth ventricle. The floor of the IV ventricle is flanked by the medial vestibular nuclei and the longitudinal medial fascicle (LMF) on both sides. The LMF is an important descending pathway that influences postural control.

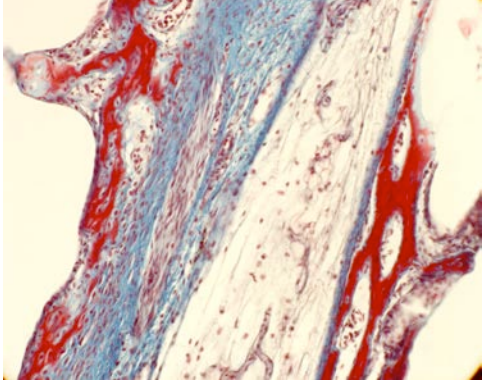
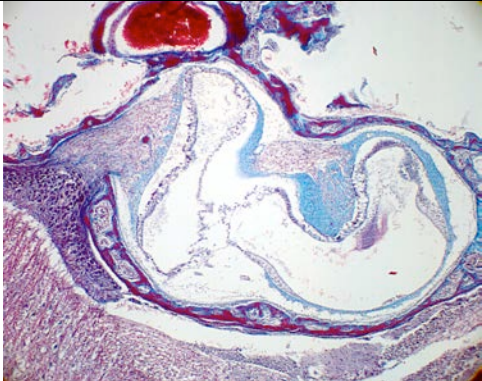
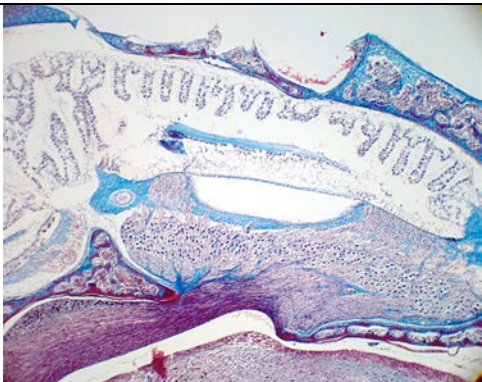
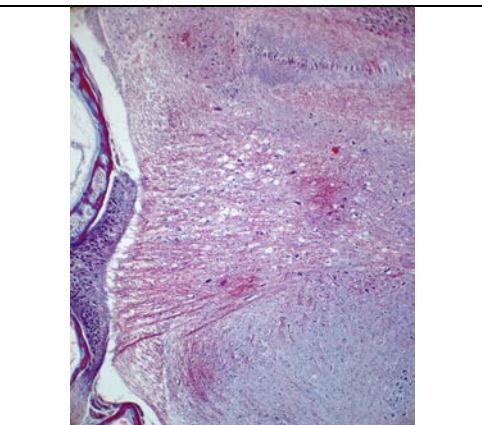


CDF-SLM-1019 (LABORATORY): This is a x40 objective view of the entrance of the vestibular nerve into the brain stem. Most of the bipolar neurons appear well preserved. Some show cytoplasmic extraction with empty spaces between the myelin and the nuclei.

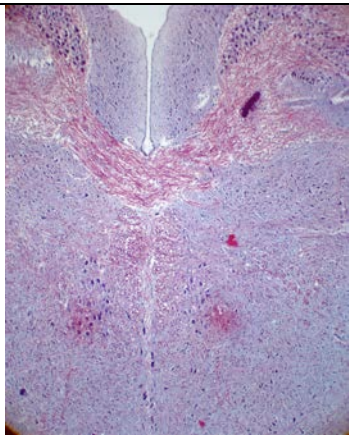
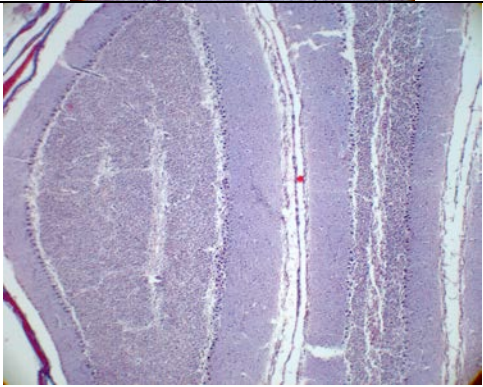
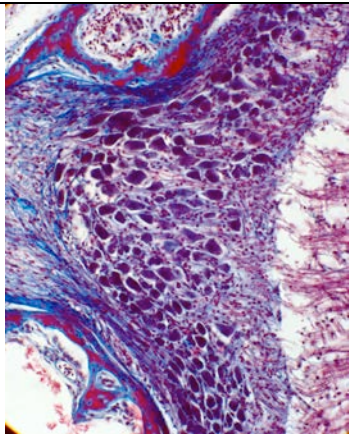
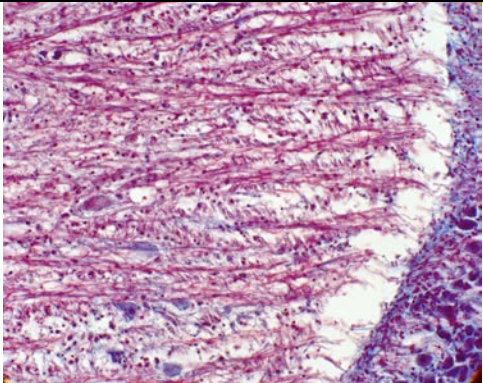
	<p>CDF-SLM-1020 (LABORATORY): Details of the sensory area of the posterior crista. The overlaying cupular membrane is seen still attached to the stereocilia and the remaining hair cells. Extensive extraction of the supporting elements is seen in between the blue hyaline cartilage that supports the crista and the cuticular lamina of the apex of the epithelia. Note excessive extraction of the afferent terminals (chalices) under hair cell type I (primary afferents). <b>Immunostaining of these structures was one of the primary objectives of the funded application and could not be fulfilled because antigenic determinants were missing.</b></p>
	<p>CDF-SLM-1021 (LABORATORY): This is a x40 objective view of the auditory vas-epithelia. The auditory branch of the eighth nerve bipolar neurons and portion of the tegmentum vasculosum (TV) to the left. The hyaline cartilage supporting the TV epithelia is still present, but the sensory epithelia supporting cells and most of the hair cells are extracted by the processing. Note also extensive vacuolization around the bipolar neurons. Contrary to the vestibular ganglion that resides outside the otic capsule, the auditory ganglion resides inside the otic capsule, and like the epithelia, it is affected greatly by the lack of fixative penetration or dilution of the fixative in extended periods of the microgravity environment imposed by space flights.</p>
	<p>CDF-SLM-1022 (LABORATORY): This is a x40 objective view of the saccule of the lab control showing improved preservation of the sensory epithelia, but still with abundant extraction of the supporting cells. The hyaline cartilage below the sensory epithelia is well preserved, as is the otoconial mass, both stained in blue. The bone is stained in red and the ciliary bundles are still seen, but separated from the otoconial membrane where they are usually tightly attached.</p>
	<p>CDF-SLM-1023 (LABORATORY): This is a x40 objective view of a cerebellar lobule showing the granular layer and Purkinje cells. The Purkinje cells are better preserved than in the synchronous control. However, there is extraction of the matrix around the Purkinje cells and no clear recognizable afferent nerve endings can be seen, which are usually populated in This is a rea.</p>

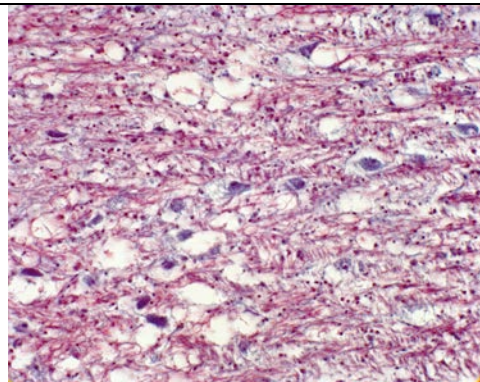
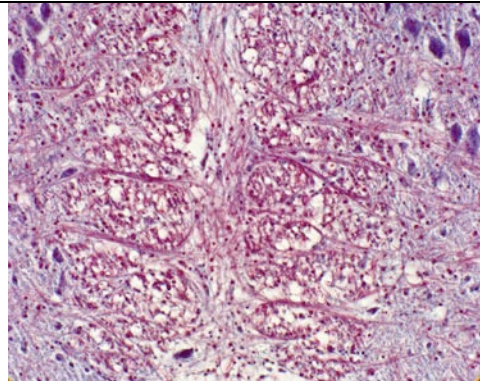
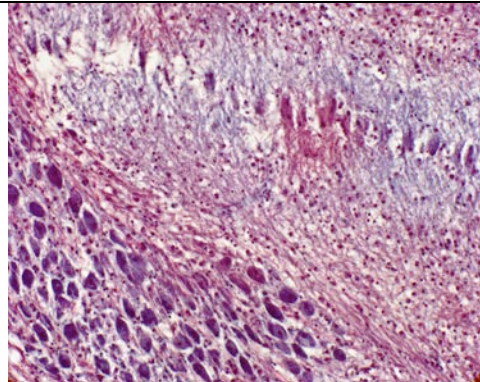
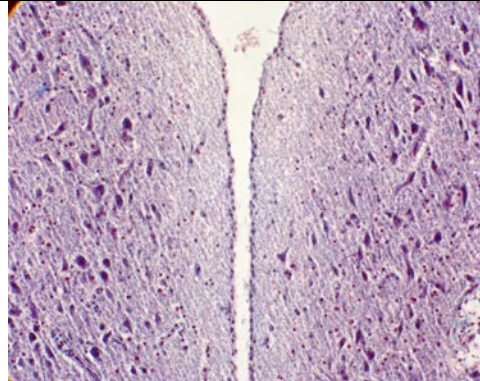


	<p>CDF-SLM-1024 (LABORATORY): This is a x40 objective view of the superior vestibular nucleus (aggregate), and the nucleus laminaris (single layer) of the mid brain stem next to the fourth ventricle. Extensive extraction of the components outside the periphery of the neurons in both nuclei is apparent.</p>
	<p>CDF-SLM-1025 (LABORATORY): This is a x40 objective view of the choroids plexus of the lab control showing good preservation of both dark and light cells. <b>A blood vessel filled with red blood cells is evident, and common in fixation by immersion as opposed to fixation by perfusion, which is possible in standard laboratory experiments but not in space.</b></p>
	<p>CDF-SLM-1026 (LABORATORY): Is 40X objective view of the tegmentum vasculosum of the lab control showing preservation of some of the light cell cytoplasm component in nuclei. Dark cells are partially preserved but extraction of all the components is obvious. The hyaline cartilage and bone are at the bottom of the structure. The TV is a highly vascularized structure that produces the endolymph of the avian ear.</p>
	<p>CDF-SLM-1027 (LABORATORY): This is a x40 objective view of a temporal muscle showing good preservation of the myofibrils because the muscle is outside the skull and are exposed to the fixing solution first. <b>The early fixation of muscle, feathers, etc., exhausts the fixative and the solution that reaches the sensory cells inside the bony capsule that protects the inner ear membranous structures is very diluted and probably of an unbalanced pH and osmolarity.</b> The cartilage in blue and the mature bone in red are also well preserved. Consequently, muscle, bone and cartilage will remain as a good source of material to study in future experimentation utilizing this fixation protocol in space.</p>

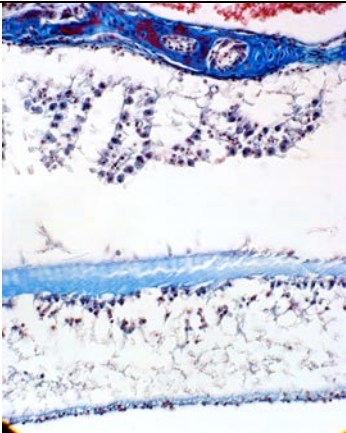
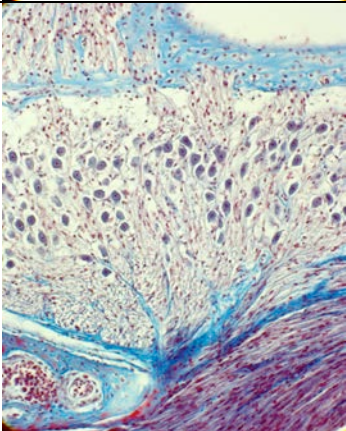
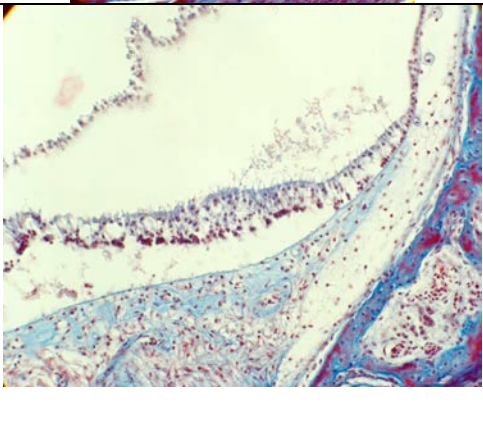
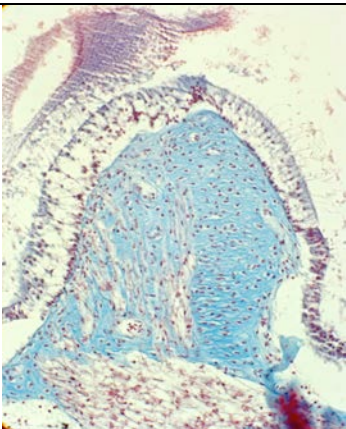
	<p>CDF-SLM-1028 (LABORATORY): This is a x40 objective view of the cartilaginous and osseous structural lining the middle ear cavity of the quail. As described in previous images, the cartilaginous, the mature bone and the fibrous components of This is a rea will continue to serve as a good source of morphological and descriptive work because they were better preserved in the soft tissues in the inner ear, they are filled with fluids and protected by hard bone.</p>
	<p>CDF-SLM-1029 (LABORATORY): This is a x10 objective view of a flight specimen showing the entire area of the convergence between the utricle and superior semicircular canal. The otic capsule and cartilaginous structure surrounding the membranous labyrinth of the ear are intact, but the epithelial components of the inner ear membranous structures are extracted, indicating poor fixation and extraction during processing. It can be surmised that if this type of extraction occurs in specimens that are fixed in the manageable conditions of 1.0g earth laboratory, extraction of specimens that have been fixed with a limited fixing solution volume, drastic changes in temperature and dilution of the solution will be worse for flight specimens.</p>
	<p>CDF-SLM-1030 (LABORATORY): This is a x10 objective view of laboratory specimens showing the auditory organ (lagena). The epithelial components of the sensory area are extracted and the tegmentum vasculosum has dark cells, but very few light cells remain intact. The otic capsule with mature bone (red) and cartilaginous structures (blue) are evident throughout. The bipolar auditory neurons form a compact ganglion.</p>
	<p>CDF-SLM-1031 (FLIGHT): This is a x10 objective view of a flight specimen showing the entrance of the vestibular nerve and ganglion to the brain stems, where spaces are seen from tangential neurons that were extracted during processing. The spaces correspond to extracted neurons due to poor fixation or to insufficient concentration of the fixative. The nucleus laminaris can also be seen in this view as well as portion of the medial vestibular nucleus.</p>

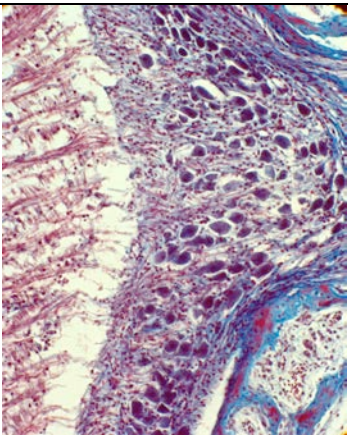
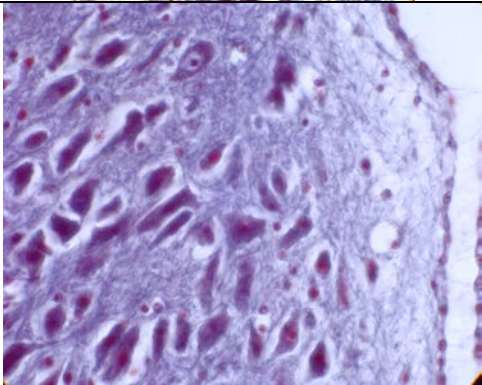
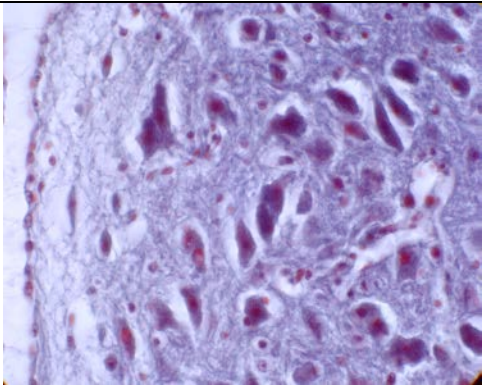
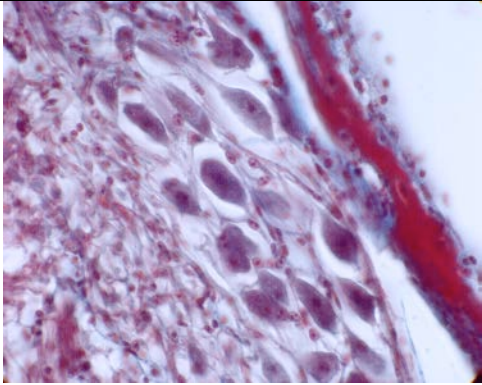


	<p>CDF-SLM-1032 (FLIGHT): This is a x10 objective view of the mid-section of a flight specimen showing both sides of the medial descending fascicle, crossing fiber interconnecting the nucleus laminaris, and portion of the medical vestibular nucleus. The floor of the IV ventricle marks the limit of the crossing fibers for the vestibular nuclear complex.</p>
	<p>CDF-SLM-1033 (FLIGHT): This is a x10 objective view n showing the cerebellar lobules with layers of Purkinje cells intercalated with granular and vestibular structure. There is separation of the various cell layers and shrinkage of the Purkinje cells. For topographical anatomical evaluation at the macro level, the preparation is sufficient, but it lacks details and antigenic determinants for immunohistochemical analyses.</p>
	<p>CDF-SLM-1034 (FLIGHT): This is a x40 objective view of flight specimen showing the vestibular ganglion of the eighth nerve. Note that most neurons stained dark red and none stained blue. These vestibular neurons are generally less basophilic than shown here, and for the above reason stain redder in with trichrome solution used. <b>A primary objective view of the funded application was to evaluate these neurons with immuno markers, but there was not expression of any of the molecules proposed.</b> The cartilage and the bone are clearly seen surrounding either side of the ganglion. Thus, there have been changes of normal cellular components leading to altered staining patterns (ratio of basophilic to acidophilic components), extraction of molecules from a loosely cross-linked cytosol, and lack of immunostaining due to absence of antigenic markers to bind to the antibodies proposed.</p>
	<p>CDF-SLM-1035 (FLIGHT): This is a x40 objective view of a flight specimen showing the edge of the vestibular ganglion and fibers from it connecting to the brain stem. A few neurons are seen in this portion of the tangential nucleus of the vestibular complex. Several large blue neurons are visible at the bottom of the frame, and empty spaces that correspond to extracted neurons due to poor fixation are evident.</p>

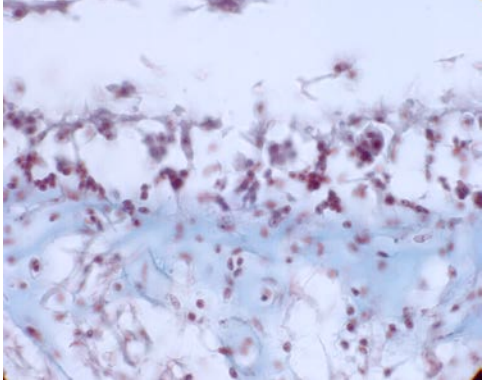
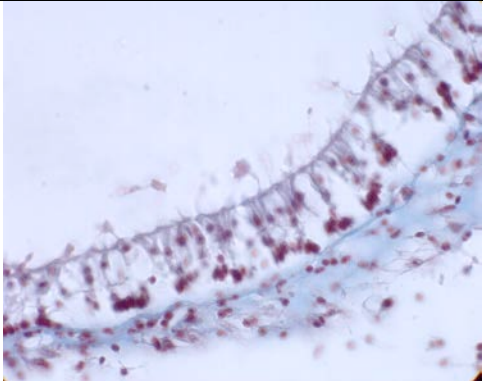
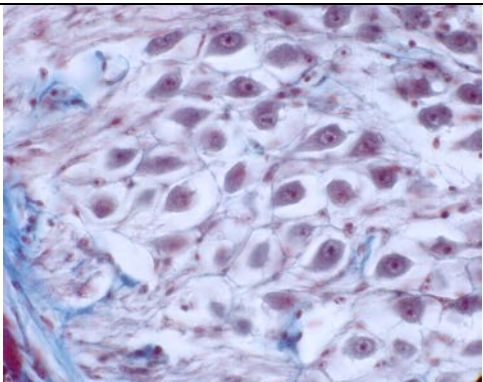
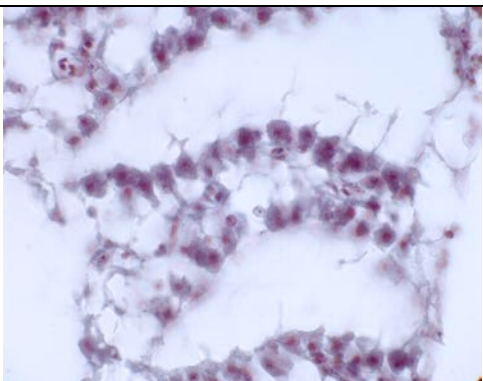
	<p>CDF-SLM-1036 (FLIGHT): This is 40x objective view of same area shown above, but deeper into the tangential nucleus area showing vacated spaces corresponding to dead and extracted neurons. Some shrunken and extremely basophilic neuronal cell bodies remains intermingled with the neuropil.</p>
	<p>CDF-SLM-1037 (FLIGHT): This is a x40 objective view of a flight specimen showing the longitudinal-medial fascicles symmetrically located in the mid-brain area of the vestibular nuclear complex to the either side of the Medial Longitudinal Fascicle (MLF). These axons belong to neurons of the sixth cranial nerve.</p>
	<p>CDF-SLM-1038 (FLIGHT): This is a x40 objective view of a flight specimen showing the nucleus laminaris and portions of the nucleus magnocellularis (lower left) of the auditory complex. Note the extraction of the neurons along the nucleus laminaris and change in coloration due to modification of the cellular components due to poor fixation. In the center, several nucleus laminaris neurons are shown in red, whereas generally, they are blue. In addition, the magnocellularis neurons are shrunken.</p>
	<p>CDF-SLM-1039 (FLIGHT): This is a x40 objective view of flight specimen showing portions of the fourth ventricle and contralateral medial vestibular nuclei. Neurons appear smaller than usual, and there is some extraction around cell bodies. This is an enlargement of CDF-SLM-1032.</p>

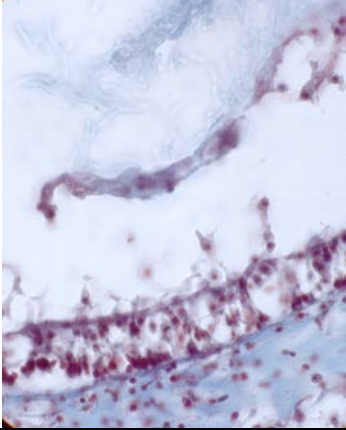
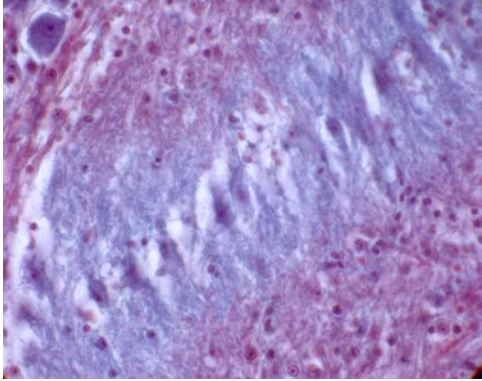
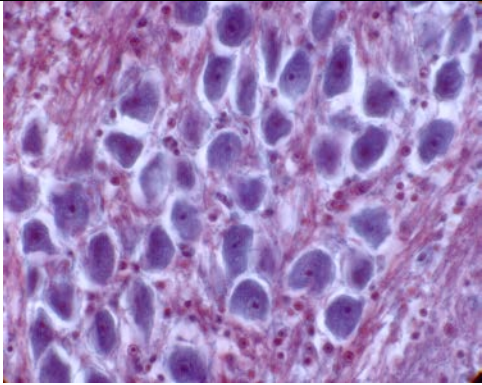
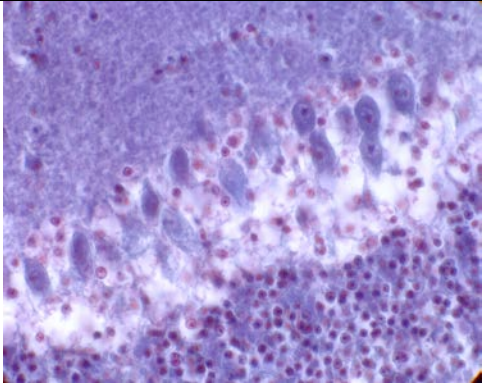


	<p>CDF-SLM-1040 (FLIGHT): This is a x40 objective view of flight specimen showing the basal membrane of the basilar papilla with complete extraction of the support cells. Some hair cells are still attached to the tectorial membrane (blue). The separation caused an increase in sensory epithelial size about three times its normal dimensions. The tegmentum vasculosum is tethered from the osseous spiral lamina, which has cartilaginous (blue) and mature bone (red). Notice that very few light cells have any cytoplasm remaining, and primarily there are dark cell with prominent red nuclei present.</p>
	<p>CDF-SLM-1041 (FLIGHT): This is a x40 objective view of flight specimen showing the statoacoustic ganglion or SAG, in its matrix adjacent to the nerve pile of the remainder of the auditory branch. The hyaline cartilage appears blue and neurons are dark blue with prominent brown nucleoli. The SAG is housed inside the optic capsule and its fixation is impaired even more than that of the vestibular ganglion, which was the target of the funded investigation.</p>
	<p>CDF-SLM-1042 (FLIGHT): This is a x40 objective view of the saccule of flight specimen showing total separation of the epithelia from the basal membrane and hyaline cartilage (light blue). The nuclear array of the support cells that separated from the base membrane is clearly seen. Remnants of hair cells &amp; cellular debris are also apparent as well as stereocilia. <b>The primary target organs of the investigation were the saccule and utricle (linear acceleration detectors), but clearly unavailable for the type of molecular analysis proposed.</b> The absence of an otoconial matrix suggests poor fixation and extraction of components from the endolymphatic space.</p>
	<p>CDF-SLM-1043 (FLIGHT): This is a x40 objective view of a flight specimen showing the main component of the superior crista. The hyaline cartilage is prominent (blue), and the mid-portion of the support epithelia has been removed from the sensory area. The stereocilia are prominent and still attached to portions of the mucopolysaccharide-rich cupula apparent above the crista.</p>

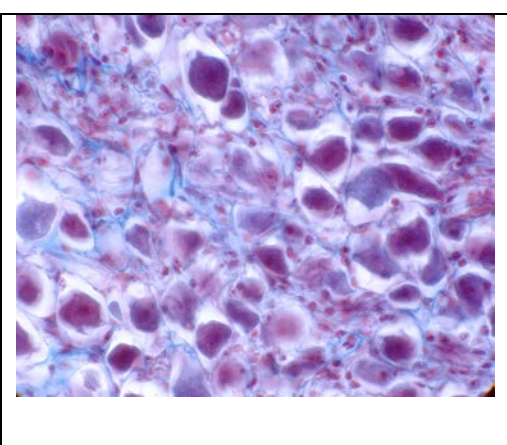
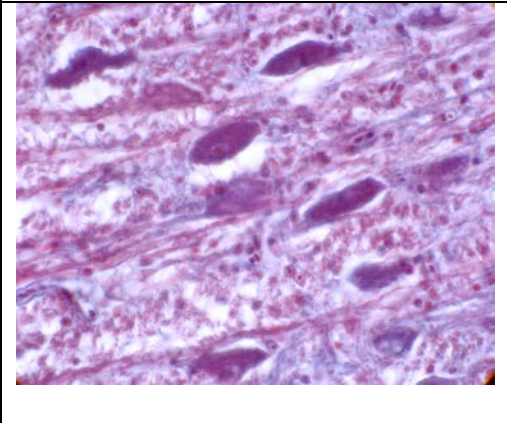
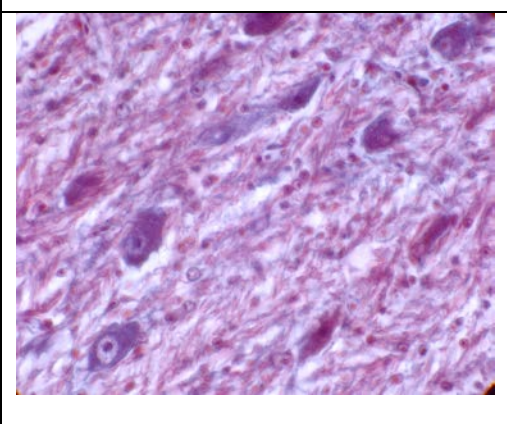
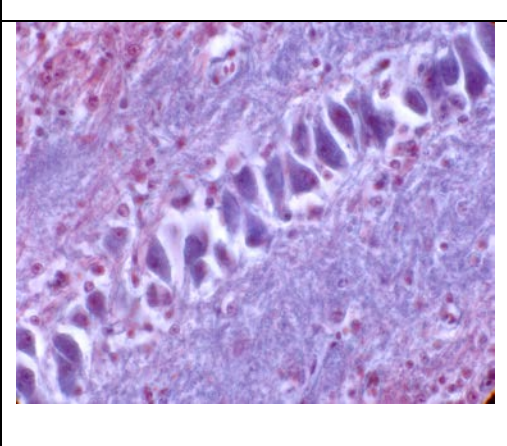
	<p>CDF-SLM-1044 (FLIGHT): This is a x40 objective view of the vestibular nerve ganglion with bipolar neurons at the entrance of the brain stem (the vestibular ganglion is housed outside the optic capsule. The neuropil stained red in the main portion of the tangential nucleus, where the peripheral neurons first make contact or synapse. Bipolar neurons appear smaller than usual, with prominent red nucleoli and excessive blue tint, which generally indicates extraction of cytoplasmic and nuclear components. Shrinkage of the neurons is apparent as well, and they all lack the normal appearance that is possible with good fixation shown in the introduction.</p>
	<p>CDF-SLM-1045 (FLIGHT): This is a x100 objective view of a synchronous control showing neurons of the medical vestibular nucleus, adjacent to the fourth ventricle wall and separated from the cerebral spinal fluid by the ependymal layer. Most of the neurons are shrunk, and retracted spaces between the neurons and the cerebellar matrix are apparent. Also notice the highly eosinophilic nuclear and nucleolar complexes.</p>
	<p>CDF-SLM-1046 (FLIGHT): This is a x100 objective view of the contralateral medial vestibular nucleus of the same flight specimen showing shrunk neurons and retracted spaces around the neurons due to extraction and poor fixation. The typical distinction between tissue groups that a trichrome stain permits is not very clear.</p>
	<p>CDF-SLM-1047 (FLIGHT): This is a x100 objective view of a flight specimen showing neurons of the vestibular ganglion with shrinking of the body. Distinction between the nucleus and cytoplasm of each neuron is more obvious than in the brain stem, probably because the fixing solution can reach the hollow space where the vestibular nerve is housed before being diluted by the dense cerebral parenchyma. Nevertheless, there not immunoreaction for antibodies proposed</p>

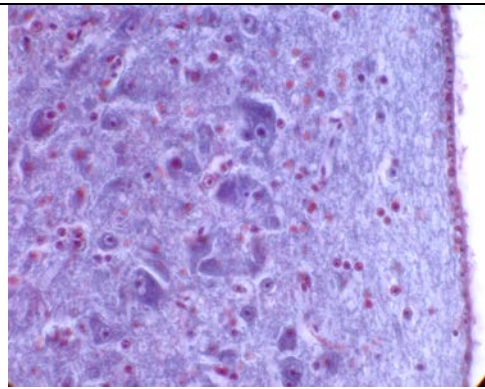
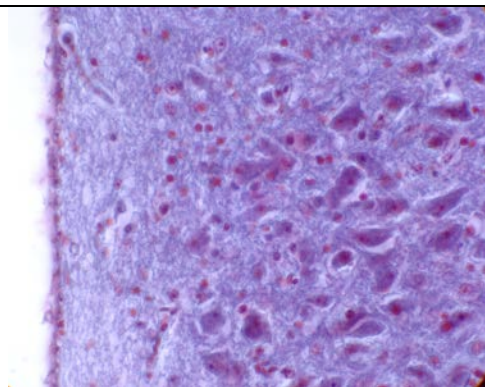
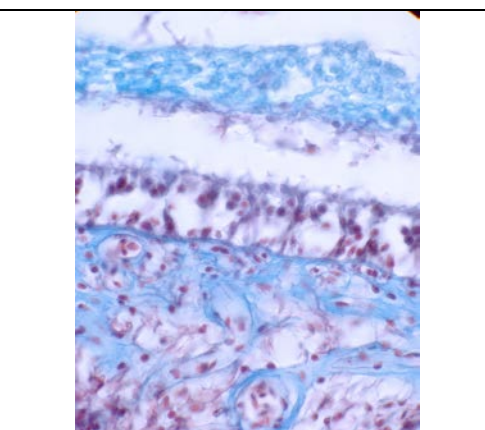
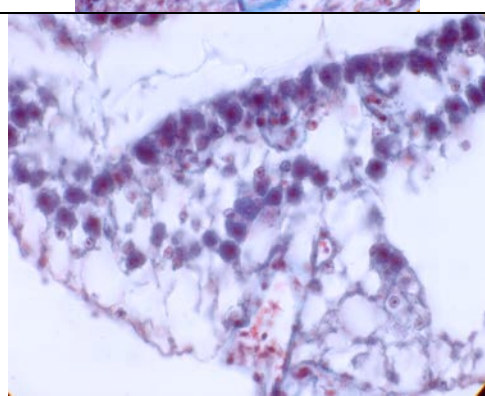


	<p>CDF-SLM-1048 (FLIGHT): This is a x100 objective view of portion of the saccular structure showing a few remaining hair cells still attached to the apical portion of the structure. Some afferent fibers surrounding more than one hair cell nucleus are apparent, but the entire supporting structure of the epithelia has been extracted and the basal membrane is disorganized to the point that it is impossible to distinguish it from the remaining hyaline cartilage (blue).</p>
	<p>CDF-SLM-1049 (FLIGHT): This is a x100 objective view of the utricular macula showing hair cells still attached to the cuticular plate and stereocilia, but no support cell. The hyaline cartilage is seen clearly in blue. <b>The utricle was one of the target structures for the funded work, but with the resulting morphology seen in this no useful data was possible, despite quite lots of efforts put into collection, preparation, and evaluation of the material.</b></p>
	<p>CDF-SLM-1050 (FLIGHT): This is a x100 objective view of the statoacoustic ganglion (auditory neurons) of the same specimen showing excessive retraction of the cytoplasm, leaving a halo around neurons. Not only was the micro appearance of the neurons bad, worse yet there was no immunohistochemical reaction for any of the antibody proposed. <b>Compare this preservation to that possible under controlled conditions at 1.0g of ground laboratories as illustrated at the beginning of the report. Wax processing yield extraction, but as an exception not as rule.</b></p>
	<p>CDF-SLM-1051 (FLIGHT): This is a x100 objective view of the tegmentum vasculosum showing the arrangement between dark and light cells. Most of the cytoplasm of the light cell has been extracted. <b>Dark cells remain because their cytoplasm has dense cytoskeletal framework that help to hold the content in place even when fixation is poor.</b></p>

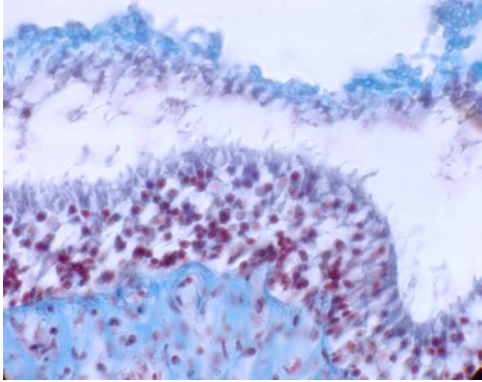
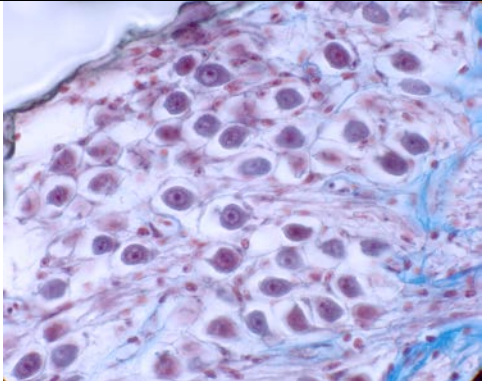
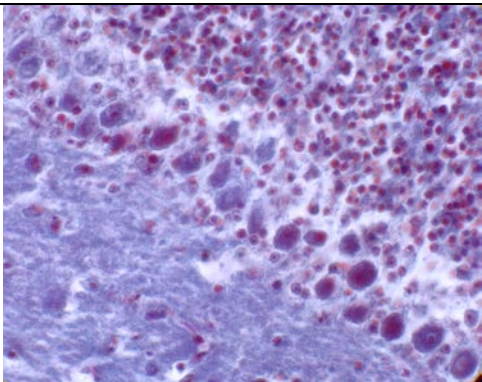
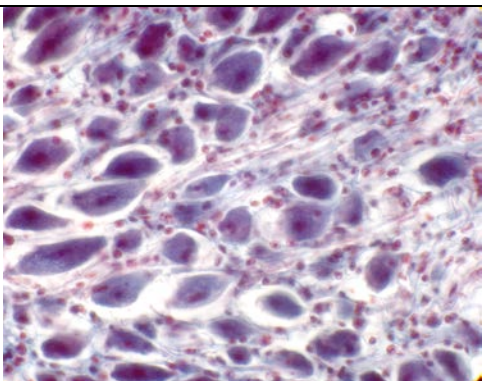
	<p>CDF-SLM-1052 (FLIGHT): On the contralateral saccule of the same specimen as shown above, x100 objective, the apical area of the sensory epithelia has been lifted, and is attached to the otoconial membrane. Note that the otoconial membrane has been reduced to a non-mineral mass, because the otoconia were extracted during extended immersion of the diluted fixative solution. The basal membrane is intact and the hyaline cartilage is obvious.</p>
	<p>CDF-SLM-1053 (FLIGHT): This is a x100 objective view of the nucleus laminaris of the flight specimen showing excessive extraction of cytoplasmic components of the neurons and lack of differentiation. Even though the nucleus laminaris is an auditory rather than a vestibular structure, it has been evaluated extensively by other avian investigators and serves to illustrate the problem of current preparation (please compare to image CDF-SLM-1059 that illustrate portion of the same nucleus with better preservation).</p>
	<p>CDF-SLM-1054 (FLIGHT): This is a x100 objective view of the nuclear magnocellularis of the same specimen showing better-preserved neurons, with slight shrinkage and retraction of the cytoplasm. This is another auditory rather than vestibular nucleus, and serves to further illustrate that poor fixation was not restricted to the peripheral and central vestibular structures.</p>
	<p>CDF-SLM-1055 (FLIGHT): This is a x100 objective view of the cerebellum with Purkinje cells bordering the granular layer. There is extensive retraction of the cytoplasm, lack of button ending and separation between the granule layer and the Purkinje cell layer. These neurons receive important input from the vestibular system to maintain normal posture and balance. <u>Consequently, Purkinje cells are always evaluated for signs of integrity of central nervous system preparations.</u></p>

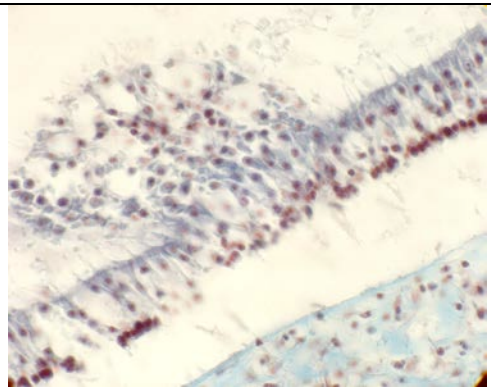
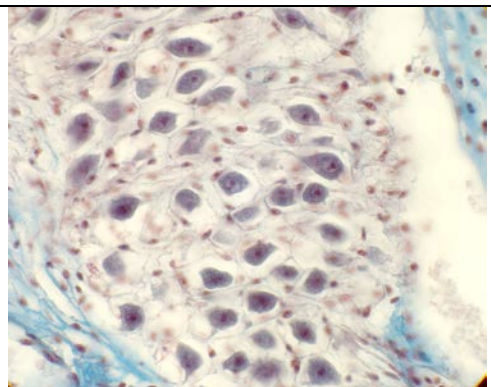
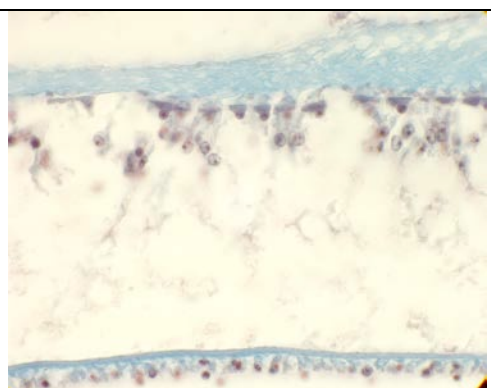
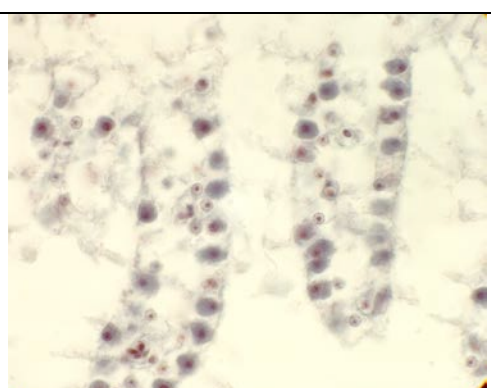


	<p>CDF-SLM-1056 (FLIGHT): This is a x100 of a flight specimen showing vestibular ganglion neurons with excessive retraction and shrinkage. There appeared to be a slightly better fixation than in previous specimens as differentiation between neuronal type is apparent by color, yet there was no antigen-antibody reaction; <b>suggesting that antigenic determinants were lost due to weak cross-linking the cytosol, or to masking of the expected epitopes by non specific denatured proteins.</b></p>
	<p>CDF-SLM-1057 (FLIGHT): Separation of tangential nucleus neurons from the cerebral matrix is apparent at 100x objective. More important however is the lack of distinction between nuclear and cytoplasmic components (both red), which is not the case in well-preserved specimens. <b>Some myelinated axons are visible, but the majority of the matrix is globular rather than fibrillar, and this is suggestive of poor fixation as well.</b></p>
	<p>CDF-SLM-1058 (FLIGHT): This is a x100 objective view of another area of the tangential nucleus tangentialis shown above in CDF-SLM-1057 with compression and chromatin damage of neurons. <b>Differences between synchronous and flight specimen at the immunohistochemical level would be helpful to evaluate changes induced by microgravity.</b></p>
	<p>CDF-SLM-1059 (FLIGHT): This is a x100 objective view of the flight specimen showing the nucleus laminaris neuron better preserved, but only small portion of the nucleus showed this preservation than in previous specimen. The majority of the nucleus surface area looked similar to image CDF-SLM-1053.</p>

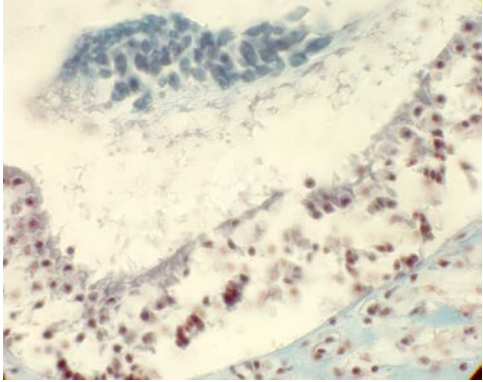
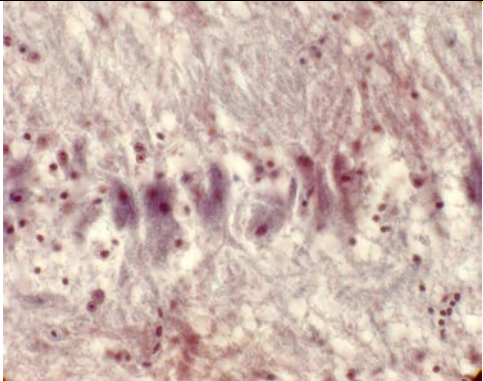
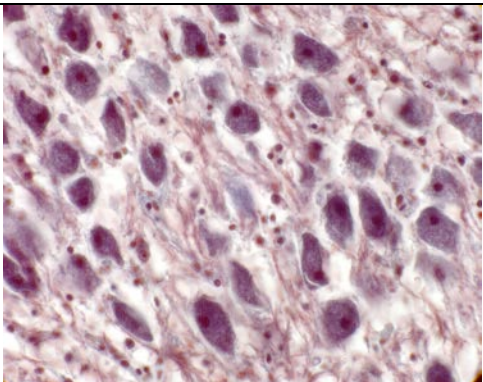
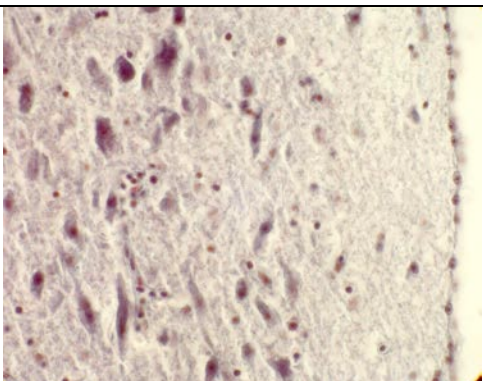
	<p>CDF-SLM-1060 (FLIGHT): This is a x100 objective view of a laboratory control showing the medial vestibular nucleus next to the fourth ventricle. There is less shrinkage of the neurons than in the synchronous control. But most neurons appeared compressed and displayed poor differentiating features between cytoplasmic and nuclear components.</p>
	<p>CDF-SLM-1061 (FLIGHT): This is a x100 objective view of a flight specimen showing contralateral (other side of figure CDF-SLM-1060) medial vestibular nucleus neurons with better fixation than those seen in the synchronous control, <b>yet there was no immunohistochemical reaction for any of the antibodies proposed as markers for afferent innervation changes that may have been induced in reduced gravity.</b></p>
	<p>CDF-SLM-1062 (FLIGHT): This is a x100 objective view of the saccule of a laboratory control showing the basal membrane intact blood vessels, portions of the central epithelia and a few recognizable hair cells, but the majority of support cells were extracted during processing suggestive of poor fixation. The otoconial membrane stains blue above the hair cells, and has separated from the stereocilia. The hexagonal shape of some otoconia units can be seen.</p>
	<p>CDF-SLM-1063 (FLIGHT): This is a x100 objective view of laboratory control showing tegmentum vasculosum dark cells and reddened nuclei of light cells where scant cytoplasmic fibrils remain.</p>

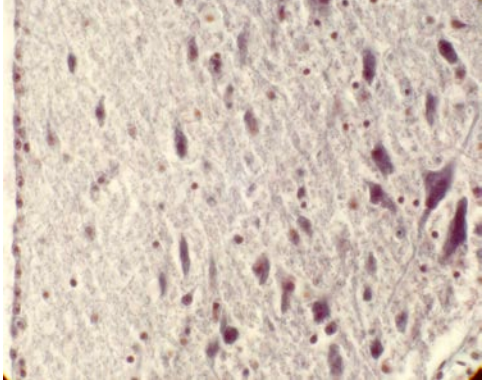
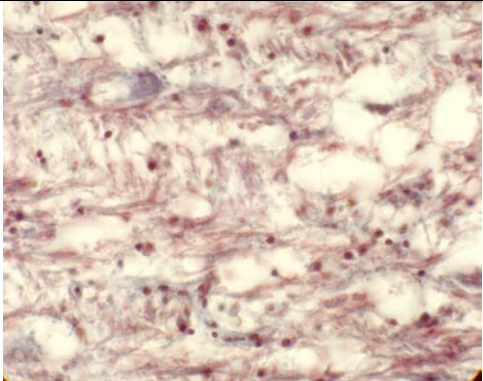
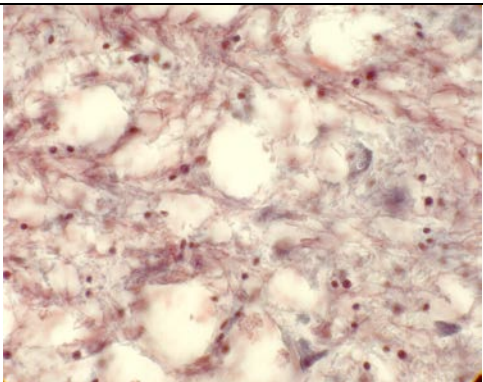
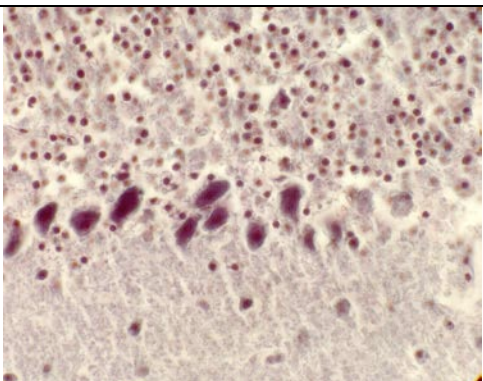


	<p>CDF-SLM-1064 (FLIGHT): This is a x100 objective view of the posterior canal crista showing hair cells, stereocilia, and portions of the utricular macular otoconia recognizable and otoconia unit, on the upper surface. There is, nevertheless, extensive extraction of the support cells matrix and only the apical portion of the hair cells remain intact. The wisps of material seen between the otoconial membrane and the hair cells are the stereociliaria bundles .</p>
	<p>CDF-SLM-1065 (FLIGHT): This is a x100 objective view of the laboratory control statoacoustic ganglion neurons showing retraction of the cytoplasm and extraction. <b>No immunohistochemical reaction for any of the calcium binding proteins proposed was obtained from any of the flight specimens.</b> Thus fulfillment of the objectives as proposed was not possible.</p>
	<p>CDF-SLM-1066 (FLIGHT): This is a x100 objective view of the laboratory control of the Purkinje cells in the cerebellar lobule showing between the granule and the cerebral cortex. There is excessive extraction of the cytoplasm of the Purkinje cells separation from the granular cell and shrinkage of the cells. There is no apparent terminal dendrite on the Purkinje cell, which is a hallmark feature of the innervation patterns in This is a rea.</p>
	<p>CDF-SLM-1067 (FLIGHT): This is a x100 objective view of a flight embryo showing the vestibular neurons of the ganglion. There is overall extraction of the ganglion matrix and lack of distinguishing features between the cytoplasm and the nucleus of each neuron. <b>It is as if the cytoplasmic content was liquefied and congealed. The overall impression is that of pyknotic nuclei seen in apoptotic cells.</b></p>

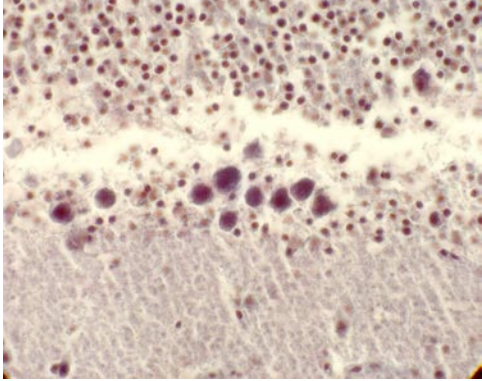
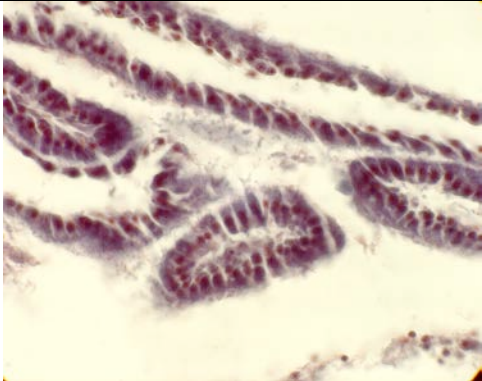
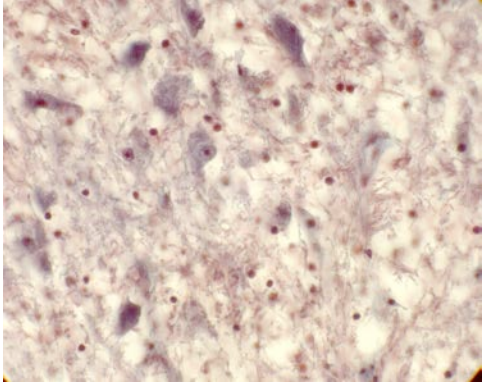
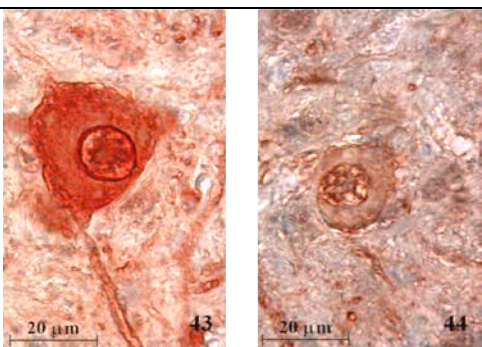
	<p>CDF-SLM-1068 (FLIGHT): This is a x100 objective view of the flight specimen showing portions of the saccule with epithelial expansion into the endolymphatic space. The support cell nuclear layer is evident, and has separated from the basal membrane. Stereocilia are seen, but no otoconial membrane is evident.</p> <p><b>Please compare this preservation to that shown at the beginning of this report. Preservation that is possible under the controlled conditions of earth laboratories.</b></p>
	<p>CDF-SLM-1069 (FLIGHT): This is a x100 objective view of a statoacoustic ganglion of a flight sample showing excessive shrinkage of the neuron and retraction of the cytoplasm. The coagulated matrix also shows signs of extraction, but the neuronal outline is still visible and demarcated by the Schwann's cells.</p>
	<p>CDF- SLM-1070 (FLIGHT): This is a x100 objective view of the auditory sensory epithelia (basilar papilla) showing the intact basal membrane, but otherwise missing support cells. The basal membrane, which is made mostly of mucopolysaccharides, retained its integrity and is visible at the bottom of the panel. A few short hair cells apices were pulled away from the epithelia due to poor fixation and can be seen as small conical structure attached to the tectorial membrane (blue).</p>
	<p>CDF- SLM-1071 (FLIGHT): This is a x100 objective view of a flight specimen showing the tegmentum vasculosum with excessively extracted epithelial components. Some of the dark cells remained, but the majority of the light cells have been extracted, including the nuclei.</p>



	<p>CDF- SLM-1072 (FLIGHT): Portion of the saccule flight specimen at x100 showing extraction of the sensory epithelia, remnants of fibrillar cuticular plates, and portions of the largest otoconia are seen in the otoconial matrix. The hexagonal shape of some otoconia crystals is recognizable (<b>the saccule contains very large otoconia that tend to fare better than small otoconia in cases of poor fixation</b>).</p>
	<p>CDF- SLM-1073 (FLIGHT): This is a x100 objective view of the nucleus laminaris of the flight specimen showing retraction of neuronal components, separation of a matrix and fusion of neurons</p>
	<p>CDF- SLM-1074 (FLIGHT): This is a x100 objective view of the nuclear magnocellularis of the flight specimen showing better - preserved neurons, but shrunken and extraction of the cerebral parenchyma.</p>
	<p>CDF- SLM-1075 (FLIGHT): This is a x100 objective view showing the medial vestibular nucleus adjacent to the fourth ventricle. The ground substance of the brain appears extracted and poorly fixed.</p>

	<p>FIGURE 76 (FLIGHT): Contralateral (opposite side to CDF-SLM-1075) medial vestibular nucleus adjacent to the fourth ventricle showing similar architecture, with overall extraction of a brain matrix.</p>
	<p>CDF- SLM-1077 (FLIGHT): This is a x100 objective view of the same specimen above showing extraction of the tangential nucleus neuron. This complete extraction of neurons could not be reconciled with the goals of the application and in particular with vestibular neurons and fiber changes</p>
	<p>CDF- SLM-1078 (FLIGHT): This is a x100 objective view of a tangential nuclear area showing additional extraction of neurons in the remnant of the cell body of certain neurons.</p>
	<p>CDF- SLM-1079 (FLIGHT): This is a x100 objective view of a cerebellar lobule showing the Purkinje cells in between the granular layer and the brain cortex. Notice the severely shrunken neurons with lack of cytoplasmic extension that characterize well fixed Purkinje neurons in well-preserved specimens. The nuclei appear lifted from the cells cytoplasm suggestive of matrix disorganization..</p>



	<p>CDF- SLM-1080 (FLIGHT): This is a x100 objective view of another area of the Purkinje cell layer sandwiched between the granular and cerebella cortex, showing extensive extraction of the cytoplasm, extraction of the matrix, and extraction of the granular cell layer. Of importance is that purkinje cell in this preparation lack contacts with other cells or fibers (See material shown in the introduction).</p>
	<p>CDF- SLM-1081 (FLIGHT): This is a x100 objective view of the choroid plexus in the fourth ventricle of the flight specimen showing intact preservation of the dark and light cells. This is probably due to the fact that the fixative first penetrates into the ventricles before it is absorbed by the choroid plexus cells, before it can reach the cerebral cortex.</p>
	<p>CDF- SLM-1082 (FLIGHT): This is a x100 objective view of the medial vestibular nuclei showing severely shrunken neuron, extensive extraction of the cerebral matrix and lack of glial cells cohesiveness that is always seen in well specimens as illustrated in the image below taken from a brain of a hatchling fixed with similar technique used for the quail samples. The red color is positive reaction for S-100 beta a protein that is highly expressed in neurons of the peripheral and central vestibular pathways.</p>
	<p>CDF- SLM-1083 (NORMAL GROUND): This is a x100 objective view of the cerebral parenchyma of a normal chicken brain fixed on a earth laboratory under optimal conditions and immunostained with antibody to S100beta, one of the afferent markers proposed for the MIR-SLM study. Similar to the quail samples shown above, this chick brain was embedded in paraffin wax, but note minimal extraction of the cerebral matrix, intact projections from the neurons at left and glial at right, and clear demarcation between the cytoplasm and the nuclei of each cells.</p>